CHAPTER I
INTRODUCTION

Modern intensive animal production systems frequently introduce animal stress and consequently reduce protective immunity to diseases (Sasitorn and Nitaya, 1998). Enteric Colibacillosis disease caused by Enterotoxigenic strains of *Escherichia coli* (ETEC) is one of the most important causes of postweaning and nursery diarrhea in pigs. This diarrhea is responsible for extremely high economic losses due to high mortality and morbidity, decreased growth rate and cost of medication. A recent survey revealed that diarrhea represented 11% of all post-weaning piglet mortality and ETEC diarrhea is the most common enteric disease in piglets, accounting for 50% of the 10 million piglets that die annually worldwide (Owusu-Asiedu et al., 2003).

There is evidence that both pathogenic and commensal *Escherichia coli* (*E. coli*) in pigs may serve as a reservoir of being disseminated to human through food chain. In the U.S., Centers for Disease Control & Prevention (CDC) reported that *E. coli* O157:H7 has emerged and killed 50 to 100 people each year (Bette, 2001). An investigation showed that ETEC has the potential to cause large foodborne outbreaks of acute gastroenteritis (Beatty et al., 2006).

Previously, food-borne illness reports have shown that ETEC were isolated from 13% of childhood cases of 1,230 children under 5 years of age with diarrhea in Thailand (Rasrinual et al., 1988). An study of infection with inherently pathogenic *E. coli* strains; urinary tract infection with extended-spectrum beta-lactamases (ESBLs)-producing *E. coli* were isolated from patients with community-acquired urinary tract infection (UTI) at Songklanagarind Hospital, Hat Yai, Thailand from July 2003 to January 2004. ESBLs producing *E. coli* were detected in six of 107 (6%) urine isolates (Tunyapanit and Pruekprasert, 2006).

Over recent decades, antibiotics have been widely formulated of animal feeds. They have been used at low levels for growth promotion purposes, at median levels for disease prevention and at therapeutic levels for the treatment and control of disease (Mercer et al., 1971). A surveyed of utilization of antibiotics growth promotion level in farms demonstrated a definite association between types of antibiotics supplied to the animals and resistance phenotypes of *E. coli* from feces. The results of this survey suggest that a high incidence of resistant organisms does exist in animals being exposed to continuous levels of antibiotics (Mercer et al., 19871).

In addition to antibiotics, antiseptic have been also widely used in food animal production for a long time. An antiseptic is a biocide that destroys or inhibits growth of microorganisms in or on living tissue. Some antiseptics have also formulated in animal feeds for growth promotion purposes, for example; copper sulfate ($CuSO_4$) and zinc oxide (ZnO). A supplemental zinc oxide at concentration of 1,500-2,000 ppm had improved postweaning pig performance and its combination with an antibacterial agent resulted in additional performance improvements (Hill et al., 2001). Copper sulfate is a common feed supplement to pigs in Denmark where its use has been the most significant in piglets which have been fed copper, as a growth promoter, at a concentration of 165 ppm (Hasman and Aarestrup, 2002).

A administration of N-alkyl dimethyl benzyl ammonium chloride in drinking water at a concentration of 50 ppm has been shown to reduce clinical signs of infectious disease significantly ($P < 0.05$) and at the concentration of 100 ppm was able to eliminate bacterial pathogens *in vitro* e.g. *E. coli* and *Salmonella* (Huberman et al., 2005).

Just like antibiotics, antiseptics have been extensively used in food-producing animals giving rise to the potential for bacteria to develop resistance to antiseptics and cross-resistance to antibiotics has become an increasing concern. The long term use of the antiseptics at concentration under or sub-therapeutic levels may trigger the adaptive response and or mutation either on chromosomal locus or resistant gene on a plasmid providing future potential for the stable antiseptic resistant gene from strain to strain and between bacterial species (Threfall et al.,1994; Purushothaman and Venkatesan,1993).

Strong evidence of cross-resistance was observed in biocides, particularly triclosan, used in home cleaning and hygiene products. *E. coli* that had adapted to develop triclosan resistance also showed elevated cross-resistance to chloramphenicol, erythromycin, imipenem, tetracycline and trimethoprim. This cross-resistance was associated with development of benzalkonium chloride resistant which cause cross-resistance to ampicillin, amoxicillin, chloramphenicol, imipenem, tetracycline and trimethoprim (Braoudaki and Hilton, 2004).

Haquinol is an antiseptic, which is chemically classified in the group of Chloro-8-Hydroxyquinoline (British Medical Journal, 1961). It was first synthetic for using as feed premix by Squibb and Sons Limited, Middlesex, UK. Mode of action of Halquinol is to inhibit function of enzyme RNA polymerase, thus interferes both RNA and protein synthesis of bacterial organism (Fraser and Creanor, 1975).

In Thailand, Halquinol has been commercial available over the past 30 years. This antiseptic has been registered with Food and Drug Administration (FDA) of Thailand for prevention and treatment of scour associated with *E. coli* and *Salmonella* infection in weaning and fattening pigs. Halquinol is an antibacterial agent of choice in Colibacillosis treatment in pigs. Between 2002-2007 the annual volume of Halquinol usage was recorded at 130,000 kilograms representing approximately 10% of total Thailand medicated pig feed market by value (Chitaroon, 2009). Halquinol has been using by addition to finished feed of nursery pigs at concentration of 120 ppm or 600 ppm consecutively for a period of 7 days or 7-10 days, respectively (Kaul and Lewis, 1965).

Halquinol is an antiseptic used widely and continuously, in the feed of commercially produced pigs. As a result, and because several scientific studies have established the occurrence of cross-resistance between antiseptics and antibiotics, the possibility increasing of cross-resistance between Halquinol and antibiotics is hypothesized. Despite this widely use, they are few studies on susceptibility of *E. coli* to Halquinol and the existing available publications are relatively old (Foster and Duggan, 1974, Cosgrove et al., 1981). There has never been a study to determine the existence of cross-resistance between Halquinol and antibiotics in bacteria.

Objective in this *in vitro* study is to test this hypothesis by examining the susceptibility of non Halquinol-exposed and Halquinol-exposed *E. coli* to Halquinol and antibiotic-resistance pattern. The objective of the study will also determine the possibility of cross-resistance between Halquinol and antibiotics in *E. coli* and to test the transferability of encoding Halquinol-resistant gene(s) in the *E. coli* isolated from pig to other bacteria.

The data from this study can be used for risk analysis of Halquinol-resistance and be used as part of antibiotic resistance monitoring in Thailand. It also can provide for the implementation of prudent use when using antiseptics in a similar to antibiotics.